

PCT/CA

03 01899

FEBRUARY 2004 13-02-04

REC'D 10 MAR 2004

WIPO

PCT

PA 1102216

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

December 08, 2003

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/430,690

FILING DATE: December 04, 2002

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)



By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

H. L. Jackson
H. L. JACKSON
Certifying Officer

BEST AVAILABLE COPY

DEC. 4. 2002 12:28PM

SWABEY OGILVY MTL 514 288 8389

NO 8333 2420

A/Pa

12/04/02

1c933 U.S. PTO

1c933 U.S. PTO
069036/09
60/430690

Please type a plus sign (+) inside the box →

Approved for use through 01/31/2001. OMB 0651-0037
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
OMB control number

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)				
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)		
Nicolas Claude Pierre Eric	BEAUDET DUPONT LEMIEUX SIMARD	Montréal, Québec, Canada 7, rue de Fermont, Blainville, Québec, Canada, J7B 1L6 97, Des Chênes, Ste-Thérèse, Québec, Canada, J7E 4Z2 585 de Largentière, App. 1, Laval, Québec, Canada H7N 4A1		
<input type="checkbox"/> Additional inventors are being named on the separately numbered sheets attached hereto				
TITLE OF THE INVENTION (280 characters max)				
AN EXOPOLYSACCHARIDES DELIVERY SYSTEM FOR ACTIVE MOLECULES				
Direct all correspondence to:		CORRESPONDENCE ADDRESS		
<input checked="" type="checkbox"/> Customer Number		020988		
OR		Type Customer Number here		
<input type="checkbox"/> Firm or Individual Name		020988		
Address		PATENT AND TRADEMARK OFFICE		
Address				
City		State	ZIP	
Country		Telephone	Fax	
ENCLOSED APPLICATION PARTS (check all that apply)				
<input checked="" type="checkbox"/> Specification Number of Pages		22	<input type="checkbox"/> Small Entity Statement	
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets		3	<input type="checkbox"/> Other (specify)	
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)				
<input type="checkbox"/> A check or money order is enclosed to cover the filing fee		FILING FEE AMOUNT (\$)		
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing Fees or credit any overpayment to Deposit Account Number:		19-5113	\$80.00	
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.				
<input checked="" type="checkbox"/> No.				
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are:				

Respectfully submitted,

SIGNATURE

Date

December 4, 2002

TYPED or PRINTED NAME

France Côté

REGISTRATION NO.
(if appropriate)

37,037

TELEPHONE

(514) 847-4263

Docket Number:

15468-7 USPR

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14.

[SOR.PAT.FORM 110 - 05/2000]

- 1 -

AN EXOPOLYSACCHARIDES DELIVERY SYSTEM FOR ACTIVE MOLECULES

BACKGROUND OF THE INVENTION

(a) Field of the Invention

[0001] This invention relates to exopolysaccharide delivery system of active molecules into a patient and/or increase the activity of the active molecules.

(b) Description of Prior Art

[0002] Encapsulation of bioactive compounds in natural or synthetic matrices has been extensively studied over the past decades. Advantages of encapsulation are numerous. First, it provides protection from the inactivation or degradation of the bioactive compound. Secondly, it controls the kinetics of compound release, allowing the optimization of the blood concentration profile. Third, it can also improve therapeutic indices of bioactive compounds like that described with micellar systems. This optimization diminishes the deleterious effects of bioactive compounds with short half lives. In addition, it permits a reduction in toxicity or synergize with the formulated drugs leading to a better treatment for the patient.

[0003] Many systems have been described to improve formulation of bioactive compounds. Among them we found colloidal drug delivery systems that are promising such as liposomes, microspheres, nanospheres and block copolymer micelles that increase the therapeutic index and improve the selectivity of various potent drugs (Gregoriadis G., (1995) TIBS, 13:527-537; Muller R.H., (1991) Colloidal Carriers for Controlled Drug Delivery and Targeting: Modification, Characterization and In vivo Distribution, CRC Press Inc., Florida, Kabanov A.V., Alakhov V. Y. (1997) "Micelles of Amphiphilic Block Copolymers as Vehicles for Drug Delivery" In Amphiphilic Block Copolymers: Self-Assembly and Applications edited by Alexandris P., Lindman B., Elsevier, Netherlands; Kwon G. et al. (1997) J. Controlled Release, 48:195-201; La S.B. et al. (1996) Journal of Pharmaceutical Sciences, 85:85-90; Kataoka K. et al. (1992) J. Control. Release, 24:119-132). These vehicles optimize the

- 2 -

therapeutic efficacy of drugs by preventing their rapid elimination from the body, reducing their systemic toxicity, delaying their degradation and optimizing their metabolism (Muller R. H. (1991) *supra*, Kabanov A. V., Alakhov V. Y. (1997) *supra*). In addition, they also provide for effective delivery of drugs to specific target sites (Muller R. H., (1991) *supra*) and aid in overcoming both transport limitations and defense mechanisms associated with the multi-drug resistance phenotype.

[0004] Various approaches have been developed to provide continuous delivery of various biologically active agents, and, although these have overcome some of the problems of delivering the agents, numerous problems remain such as the linearity of release, the biocompatibility of the materials used and the loading capacity.

[0005] It would be highly desirable to be provided with a natural biopolymers forming micelles, easily and inexpensively produced, enabling the delivery of an active molecule to a patient.

SUMMARY OF THE INVENTION

[0006] One aim of the invention is to use exopolysaccharides (EPS) micelles as a drug delivery system.

[0007] Another aim of the present invention is to describe a method of production of exopolysaccharides having micellar properties.

[0008] The active molecule may be lipophilic, hydrophilic, hydrophobic.

[0009] The miscellar system of the present invention is also suitable to the cosmetic industry such as in the delivery of active agents in creams, toiletries, deodorants, skin and sunscreen preparation. The micellar system of the present invention is also useful in perfumes, by stabilizing the unstable components thereof and by controlling the release kinetics of the fragrance upon application.

[0010] In accordance with the present invention there is provided a delivery system for delivery of an active molecule to a patient, the delivery system comprising a population of exopolysaccharide micelles, each micelle defining a core for containing the active molecule.

- 3 -

- [0011] The delivery system in accordance with a preferred embodiment of the present invention, wherein the exopolysaccharide is produced by lactic acid bacteria.
- [0012] The delivery system in accordance with a preferred embodiment of the present invention, wherein the bacteria is selected from the group consisting of *Lactobacillus* strain R2C2, *Lactobacillus* strain Inix, *Lactobacillus* strain Es1, *Lactobacillus* strain K2, *Candida kefir* and *Candida norvegensis*.
- [0013] The delivery system in accordance with a preferred embodiment of the present invention, wherein the active molecule is selected from the group consisting of DNA, RNA, protein, peptide, peptidomimetic, virus, bacteria, nutraceutical product and pharmaceutical agent.
- [0014] The delivery system in accordance with a preferred embodiment of the present invention, wherein the pharmaceutical agent is selected from the group consisting of analgesic, anesthetic, antibiotic, anticancer, anti-inflammatory, and antiviral.
- [0015] The delivery system in accordance with a preferred embodiment of the present invention, wherein the anticancer agent is selected from the group consisting of alkylating agents, alkyl sulfonates, aziridines, ethylenimines, methylamelamines, acetogenins, camptothecin, bryostatin, callistatin, CC-1065, cryptophycins, dolastatin, duocarmycin, eleutherobin, pancratistatin, sarcodictyin, spongistatin, nitrogen mustards, nitrosureas, antibiotics, anti-metabolites, folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate, purine analogs, pyrimidine analogs, androgens, anti-adrenals, folic acid replenisher, aceglatone, aldophosphamide glycoside, aminolevulinic acid, amsacrine, bestabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidamine, maytansinoids, mitoguazone, mitoxantrone, mopidamol, nitracrine, pentostatin, phenamet, pirarubicin, podophyllinic acid, 2-ethylhydrazide, procarbazine, PSK.RTM., razoxane, rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2, 2',2"-trichlorotriethylamine, trichothecenes, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine,

arabinoside, thiotepa, taxanes, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, methotrexate, platinum, vinblastine, platinum, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, navelbin, novantrone, teniposide, daunomycin, aminopterin, xeloda, ibandronate, CPT-11, topoisomerase inhibitor RFS 2000, difluoromethylomithine, retinoic acid, capecitabine, anti-hormonal agents that act to regulate or inhibiting hormone action in hormonal dependent cancers.

[0016] The delivery system in accordance with a preferred embodiment of the present invention, wherein the anti-hormonal agent is an anti-estrogens or an anti-androgens selected from the group consisting of flutamide, nilutamide, bicalutamide, leuprolide, and goserelin, and pharmaceutically acceptable salts, acids or derivatives thereof.

[0017] The delivery system in accordance with a preferred embodiment of the present invention, wherein the alkylating agents is selected from the group consisting of thiotepa and cyclophosphamide (CYTOXAN™).

[0018] The delivery system in accordance with a preferred embodiment of the present invention, wherein the alkyl sulfonates is selected from the group consisting of busulfan, improsulfan and piposulfan.

[0019] The delivery system in accordance with a preferred embodiment of the present invention, wherein the aziridines is selected from the group consisting of benzodopa, carboquone, meturedopa, and uredopa.

[0020] The delivery system in accordance with a preferred embodiment of the present invention, wherein the methylamelamines is selected from the group consisting of altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine.

[0021] The delivery system in accordance with a preferred embodiment of the present invention, wherein the acetogenins is selected from the group consisting of bullatacin and bullatacinone.

[0022] The delivery system in accordance with a preferred embodiment of the present invention, wherein the camptothecin is the synthetic analogue topotecan.

- 5 -

- [0023] The delivery system in accordance with a preferred embodiment of the present invention, wherein the CC-1065 is selected from the group consisting of adozelesin, carzelesin and bizelesin synthetic analogues thereof.
- [0024] The delivery system in accordance with a preferred embodiment of the present invention, wherein the cryptophycins is selected from the group consisting of cryptophycin 1 and cryptophycin 8.
- [0025] The delivery system in accordance with a preferred embodiment of the present invention, wherein the duocarmycin is selected from the group consisting of KW-2189 and CBI-TMI.
- [0026] The delivery system in accordance with a preferred embodiment of the present invention, wherein the nitrogen mustards is selected from the group consisting of chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide and uracil mustard.
- [0027] The delivery system in accordance with a preferred embodiment of the present invention, wherein the nitrosureas is selected from the group consisting of carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine.
- [0028] The delivery system in accordance with a preferred embodiment of the present invention, wherein the anti-metabolites is selected from methotrexate and 5-fluorouracil (5-FU).
- [0029] The delivery system in accordance with a preferred embodiment of the present invention, wherein the purine analogs is selected from the group consisting of fludarabine, 6-mercaptopurine, thiamiprine and thioguanine.
- [0030] The delivery system in accordance with a preferred embodiment of the present invention, wherein the pyrimidine analogs is selected from the group consisting of ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine and floxuridine.
- [0031] The delivery system in accordance with a preferred embodiment of the present invention, wherein the androgens is selected from the group

consisting of calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone.

[0032] The delivery system in accordance with a preferred embodiment of the present invention, wherein the anti-adrenals is selected from the group consisting of aminoglutethimide, mitotane and trilostane.

[0033] The delivery system in accordance with a preferred embodiment of the present invention, wherein the antibiotics is selected from the group consisting of enediyne antibiotics, aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, poffiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin.

[0034] The delivery system in accordance with a preferred embodiment of the present invention, wherein the enediyne antibiotics is selected from the group consisting of calicheamicin, dynemicin, esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores.

[0035] The delivery system in accordance with a preferred embodiment of the present invention, wherein the calicheamicin is selected from the group consisting of calicheamicin γ 11 and calicheamicin θ 11.

[0036] The delivery system in accordance with a preferred embodiment of the present invention, wherein the dynemicin is dynemicin A.

[0037] The delivery system in accordance with a preferred embodiment of the present invention, wherein the micelles are having a diameter varying from about 4 nanometers to about 400 nanometers.

[0038] In accordance with the present invention, there is provided a pharmaceutical composition comprising the delivery system of the present invention in association with a pharmaceutically acceptable carrier.

- [0039]** In accordance with the present invention, there is provided an immunomodulator composition comprising an immunomodulating amount of the delivery system of the present invention in association with a pharmaceutically acceptable carrier.
- [0040]** In accordance with the present invention, there is provided a method for delivering an active molecule to a patient comprising the step of administering the composition of the present invention to the patient.
- [0041]** The method in accordance with a preferred embodiment of the present invention, wherein the administration can be performed by a route selected from the group consisting out local, parenteral, peritoneal, mucosal, dermal, epidermal, subcutaneous, transdermal, intramuscular, nasal, oral, topical, vaginal, rectal, intra-ocular, intravenous, intra-arterial and by inhalation.
- [0042]** In accordance with the present invention, there is provided a method for inducing immunomodulation in a patient comprising the step of administering the composition of the present invention to the patient.
- [0043]** In accordance with the present invention, there is provided the use of the composition of the present invention for delivering an active molecule to a patient.
- [0044]** In accordance with the present invention, there is provided the use of the composition of the present invention for inducing immunomodulation in a patient.
- [0045]** In accordance with the present invention, there is provided a method for producing the delivery system of the present invention, comprising the step of incubating exopolysaccharide in a suitable medium for a time sufficient to form micelle.
- [0046]** For the purpose of the present invention the following terms are defined below.
- [0047]** The term "active molecule" is intended to mean, without limitations, nonpolar, lipophilic drugs, vitamins, immunosuppressants, immunoactive agents, neutraceuticals, peptidomimetics mimicking growth factors and their antagonists and immunomodulator agents

[0048] All references herein are hereby incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0049] Fig. 1 illustrates scans of pyrene dissolved in PBS, P85 and EPS;

[0050] Fig. 2 is an electronic microscopy image of pur EPS micelles; and

[0051] Fig. 3 is an electronic microscopy image of EPS micelles having 1% critical micellar concentration.

DETAILED DESCRIPTION OF THE INVENTION

[0052] In accordance with the present invention, there is provided a delivery system comprising exopolysaccharide micelles for delivering an active molecule to a patient.

[0053] Exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) are known to act as viscosifying agents in fermented foods (Yang, Z. (2000) Antimicrobial and extracellular polysaccharides produced by lactic acid bacteria: Structures and properties, PhD thesis, University of Helsinki, Helsinki, 61 pp.) and as cytokine inducers *in vitro* (Chabot, S. et al, (2001) Exopolysaccharides from *Lactobacillus rhamnosus* RW-9595M stimulate TNF, IL-6 and IL-12 in human and mouse cultured immunocompetent cells, and INF- γ in mouse splenocytes, *Lait*, 81:683-697). The rheological and immunomodulator properties of EPS are based on their monomeric composition and their assembling, conferring to those molecules a neutral to charged tri-dimensional structure that can actively interact with its environment. It was observed during the use of pyrene, an insoluble molecular probe in water, a modification of its fluorescent profile in the presence of EPS in comparison to the pluronic P85 (a tri-block copolymer known to form micelles in solution) profile in the same conditions. The temperature of formation is from room temperature to 37°C. The micelles form rapidly and were left undisturbed for a period of 16 to 24 hours to allow equilibration before fluorescence reading. The estimated critical micellar concentration (0.01% (w/v)) and the presence of a significant excimer peak show that EPS is a potential high loading drug carrier, since the excimer state is attributable to molecular stacking of pyrene in an

enclosed environment. An electronic microscopy analysis has been processed on EPS samples, showing semi-spherical structures resembling monolayer liposomes. EPS represents an easy-to-produce, and at a reasonable cost, bio-degradable polymer that is naturally derived from lactic acid bacteria (LAB) which are food-grade microorganisms with the GRAS status (Generally Recognized As Safe) (see Figs. 2 and 3).

[0054] The micellar delivery system of the present invention is able to incorporate much higher concentrations of pyrene as compared to P85 (see Examples) that will later be released slowly over time. In this way, it will prevent some of the damaging effects that high doses would induce.

[0055] The preliminary results obtained for the exopolysaccharide micellar system of the present invention reveal it to be a promising drug delivery vehicle for the delivery of various active molecules including lipophilic drugs. The small size and high *in vitro* stability of the micelles render them useful for a wide variety of biomedical applications. Their loading capacity for the very hydrophobic pyrene compound shows that it allows incorporation of several different compounds with a high degree of hydrophobicity.

[0056] The degree of non-covalent incorporation of physical entrapment of a hydrophobic drug into a micelle is determined by the partition coefficient of the drug between the micellar core and the surrounding aqueous medium. The affinity of the exopolysaccharide micelles for lipophilic compounds was assayed by the determination of the partitioning coefficient (solubilization) for the hydrophobic model compound, pyrene, between the exopolysaccharide micelles and water. This method has been previously used to determine the partitioning coefficient of pyrene between several polymeric formulations forming micelles and water and the critical micellar concentration (CMC). This fluorescence method is based on pyrene's sensitivity to the hydrophobicity of its microenvironment. This is reflected in changes in the ratio of the I_3/I_1 bands of its emission spectrum (see Fig. 1). The method requires the measurement of the I_3/I_1 ratio for pyrene in micelle solutions of various solvent mixtures. The CMC is calculated by plotting either the I_3/I_1 (aqueous) over the concentration of polymers or exopolysaccharide. This plot has a S-shape and the inflection

- 10 -

point corresponds to the CMC. Furthermore, by plotting I_3 (test solution) on I_3 (aqueous) for a range of concentration gives an idea of the capacity to solubilize the pyrene (partitioning coefficient). Finally, I_4 or I_6 which correspond to the peak of excimer (pile up of ground state and excited pyrene molecules) represents an accumulation of pyrene in an hydrophobic microdomain. By plotting I_4 or I_6 (test solution) on I_4 or I_6 (aqueous or control) for a range of concentration gives an indication about the magnitude of pyrene accumulation in hydrophobic pocket like micelles or micellar like structure. Also, the ratio I_4 or I_6 (test solution) on I_3 (test solution) represents the degree of friction in the hydrophobic domains. A high ratio is a sign of high viscosity. Finally, I_3 over I_1 from the same profile (test solution) is a measurement of the polarity of the solvent.

[0057]

The *in vitro* toxicity of the exopolysaccharide micelles was tested by incubating the micelles with a wide range of cell lines for both 24-hour and 48-hour periods. The XTT survival assays were then used to quantify the survival rates in the presence of the exopolysaccharide micelles. The capability of the exopolysaccharide micelle system to deliver a hydrophobic compound was first tested *in vitro* using pyrene as a model compound. The pyrene-incorporated exopolysaccharide micelles were incubated with B16 and Caco-2 cells over a 24-hour period. At 0, 4, 8 and 24 hours, samples of the supernatant were studied by fluorescence in order to determine the quantity of pyrene remaining in the supernatant at each time point. Likewise, at each time point, cells were isolated and their fluorescence was examined under the fluorescence microscope in order to determine if the decrease in the fluorescence within the supernatant was accompanied by an increase in fluorescence within the cells. The results from XTT survival assays found the micelles to cause little or no cell death when compared with the control wells of untreated cells.

[0058]

The exopolysaccharides are easy to use since there is no need for micelle preparation. The exopolysaccharides were dissolved in PBS pH 7.2 and incubated overnight at room temperature, which led to the spontaneous formation of the EPS micellar structure. Micellization process with exopolysaccharide is different than the conventional preparation of micelles (like P85) which is achieved by the addition of water in a dropwise fashion to a solution to form a micelle solution and the

- 11 -

micelle solution did not need to be stirred overnight and then dialyzed against milli Q distilled water using dialysis tubing with a change of water every hour for the first four hours and then every three hours for the next twelve hours like it is usually done.

[0059] The present invention describes the formation of micelles of different size composed of exopolysaccharide ranging from 4 to 400nm. The exopolysaccharide can be isolated from, but not limited to *Lactobacillus* strain R2C2, *Lactobacillus* strain Inix, *Lactobacillus* strain Es1, *Lactobacillus* strain K2.

[0060] The delivery system of the present invention is intended to be suitable for the delivery of any one of, without limitation, DNA, RNA, protein, peptide, peptidomimetic, virus, bacteria, nutraceutical product and pharmaceutical agent. The term include any pharmaceutical agents, including, but not limited to analgesic, anesthetic, antibacterial, anticancer, anti-inflammatory and antiviral.

[0061] The exopolysaccharide not only can form micelles but can act as a biological response modifying agent on cells. As shown in one example, exopolysaccharide can activate genes in keratinocytes which suggest that exopolysaccharides can be used not only as a drug delivery system but may have a synergistic effect with various drugs.

EXAMPLE 1

Extraction of EPS

[0062] EPS are extracted from biomass of a consortium of bacteria and yeast strains that include but not limited to the following ones, *Lactobacillus* strain R2C2, *Lactobacillus* strain Inix, *Lactobacillus* strain Es1, *Lactobacillus* strain K2, *Candida kefir*, *Candida norvegensis*. EPS can also be produced from the purified bacterial strain mentioned above.

EXAMPLE 2

Crude preparation and production of EPS

[0063] Biomass (either consortium or purified bacterial stain) is added to hot water (0.5 to 5% (w/v)). The temperature of the solution is brought to 95°C and agitation is applied. Dissolution of biomass is visually

- 12 -

monitored and when no further dissolution occurs the incubation is pursued for an extra hour. A primary filtration is performed to remove aggregates and debris and the filtrate centrifuged. When large volumes (over 10L) are processed, a concentration step by tangential filtration is performed before the centrifugation. The supernatant is recovered and freeze dried to yield the crude EPS. More specifically, the frozen biomass is added to hot water at a ratio of 2% (w/v). The water temperature is brought to 95°C for a 2 hour period with agitation at 250 rpm. The warm solution is filtered through cotton cloth to remove aggregates and debris. The filtrate is concentrated on 3kDa membrane by tangential filtration. The concentrate is centrifuged at 4000 x g for 20 minutes at room temperature. The supernatant is recovered and freeze dried to yield crude EPS.

EXAMPLE 3

Purified preparation of EPS

[0064]

Biomass (either consortium or purified bacterial strain) is added to hot water (0.5 to 5% (w/v)). The temperature of the solution is brought to 95°C and agitation is applied. Dissolution of biomass is visually monitored and when no further dissolution occurs the incubation is pursued for an extra hour. A primary filtration is performed to remove aggregates and debris. When large volumes (over 10 L) are processed, a concentration step by tangential filtration is performed. EPS from the filtrate are recovered by two successive precipitations with cold ethanol. The EPS are resuspended in water and freeze dried to yield pure EPS. More specifically, the frozen biomass is added to hot water at a ratio of 2% (w/v). The water temperature is brought to 95°C for a 2 hour period with agitation at 250 rpm. The warm solution is filtered through cotton cloth to remove aggregates and debris. The filtrate is concentrated on 3 kDa membrane by tangential filtration. The solution is centrifuged at 4000 x g for 20 minutes at room temperature to remove insoluble impurities. The concentrate is mixed with one volume of cold ethanol (-70°C) and incubated at 4°C for 16 hours. The solution is centrifuged at 4000 x g for 20 minutes at 4°C. The supernatant is discarded and the pellet resuspended in a minimal volume of water. The solution is mixed with one volume of cold ethanol (-70°C) and incubated at 4°C for 16 hours. The

- 13 -

solution is centrifuged at 4000 x g for 20 minutes at 4°C. The pellet is recovered, resuspended in water and freeze dry to yield pure EPS.

EXAMPLE 4

Fractionation of EPS

[0065] Pure EPS are solubilized in water and fractionated by serial passages on membrane of different molecular weight cutoff. As example, if membranes used were: 100 000, 50 000, 10 000 and 3 000 kDa, this procedure will yield fraction of EPS with the following range of molecular weight: EPS > 100 000, 50 000<EPS>100 000, 10 000<EPS>50 000, 3 000<EPS>10 000, EPS<3 000.

EXAMPLE 5

Characterization of EPS – Microscopy analysis of EPS

[0066] An electronic microscopy analysis has been processed on EPS samples, showing semi-spherical structures resembling monolayer liposomes. Micelle Size Distribution was determined by Dynamic laser light scattering (DLS) measurements on the exopolysaccharide micelles were carried out using a Brookhaven laser light scattering instrument (Brookhaven Instruments Corporation, New York, U.S.A.), with a uniphase 125 mW micro green laser at a wavelength of 532 nm at 25°C. A scattering angle of 90° was used for all measurements. The concentration of the samples ranged from 0.01 to 0.2% w/w in filtered (0.8 um filter) deionized distilled Milli Q water.

EXAMPLE 6

Measurement of critical micelle concentration (CMC) of EPS using Pyrene

[0067] Prepare a 1% solution of EPS (w/v) in PBS. Vortex for 2 minutes until EPS is well resuspended. Proceed to multiple dilutions to obtain 2 ml of 0.1%, 0.01%, 0.001% EPS solution. In parallel, prepare a series of borosilicate tubes to which 20 ul of a pyrene solution (50 uM solubilized in acetone (Sigma Aldrich cat. No. 18-551-5)) were added and allowed to dry prior to the addition of the various EPS solutions. Once dried, transfer 2 ml of the EPS solutions to be tested into the borosilicate tubes. This is called a redissolution test or solubilization or partitioning

- 14 -

between water and test conditions. Place the control tube (containing PBS only) in the cell holder set at 37°C of an Varian Eclipse fluorometer set at Excitation 340 nm, Emission 350-600 nm. Samples are incubated over time and read to obtain the partitioning coefficient and CMC measurement. CMC are calculated according to the standard protocols as described previously. The data of Table 1 show the ratio of fluorescence intensity of peak I_3 over intensity of peak I_3 from PBS solution as a function of the concentration of EPS and P85. This data show that the EPS can solubilize the insoluble probe (pyrene) as much if not more than P85 from the range 0,001% to 0,1%. At higher concentration (1%), P85 seems to keep forming conventional micelles while the EPS start to decline which is explained by a shift in the spectrum profile. This shift is indicative of excimers formation.

Table 1

ratio of fluorescence intensity of peak I_3 over intensity of peak I_3 from PBS solution in function of the concentration of EPS and P85

Concentration	P85	EPS lot 2
0,001%	1,107	1,125
0,01%	1,168	1,690
0,1%	2,564	3,989
1%	8,813	3,476

The data of Table 2 show the ratio of fluorescence intensity of peak I_6 over intensity of peak I_6 from PBS solution in function of the concentration of EPS and P85. This data show that EPS triggers a higher formation of excimer than P85.

Table 2

ratio of fluorescence intensity of peak I_e over intensity of peak I_e from PBS solution in function of the concentration of EPS and P85

Concentration	P85	EPS lot 2
0,001%	0,9619	2,241
0,01%	1,233	19,51
0,1%	2,443	60,31
1%	4,829	11,97

The data of Table 3 show the ratio of fluorescence intensity of peak I_e over intensity of peak I_m as a function of the concentration of EPS and P85. This data show that the EPS are more viscous than P85.

Table 3

ratio of fluorescence intensity of peak I_e over intensity of peak I_m as a function of the concentration of EPS and P85

Concentration	P85	EPS lot 2
0,001%	0,06239	0,1430
0,01%	0,07580	0,8293
0,1%	0,06841	1,086
1%	0,03935	0,2472

The data of Table 4 show the ratio of fluorescence intensity of peak I_3 over intensity of peak I_1 in function of the concentration of EPS and P85. This data show that both P85 and EPS are apolar media.

Table 4

**ratio of fluorescence intensity of peak I₃ over intensity of peak I₁ in
function of the concentration of EPS and P85**

Concentration	P85	EPS lot 2
0,001%	0,9271	0,9119
0,01%	0,9226	1,031
0,1%	0,9538	1,257
1%	0,9892	1,328

EXAMPLE 7**Formulation of 5FU with EPS and studies on B16 and Caco-2 cells**

Caco-2 and melanocytes B16 cells were cultured in DMEM supplemented with 10% FBS. The cells were seeded at 2×10^3 cells per well in a 96-well plate and left to rest 24 hours before EPS exposure at various concentrations and also with or without 5FU (0,001 ug/ml to 10 ug/ml). The cells were grown in a CO₂ incubator for 4 extra days. Cell survival was assessed with XTT according to manufacturer's recommendation. The data obtained show a synergistic effect between 5FU and the EPS while EPS show no or only modest toxicity.

EXAMPLE 8**Biological effects of EPS on keratinocytes**

HEKa (Cascade Biologics) cells were grown in Medium 154 supplemented with human keratinocytes growth supplement (HKGS) were exposed to various concentrations of exopolysaccharide and the RNA was isolated using TRIZOL reagent (Gibco) as per manufacturers specifications. Ten micrograms of total RNA was reverse transcribed using Superscript RT (Gibco), 500 ng oligodT primers (Gibco), and 250 ng Random Hexamer primers (Gibco) for 20 minutes at room temperature, followed by 2 hours at 42°C. The RNA was then labeled to perform gene profiling on specialized microarrays filters (DermaArray, Resgen.). Exopolysaccharides activate a variety of genes in keratinocytes that may be taken advantage of for cosmeceutical purposes.

- 17 -

[0068]

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A delivery system for delivery of an active molecule to a patient, said delivery system comprising a population of exopolysaccharide micelles, each said micelle defining a core for containing said active molecule.
2. The delivery system of claim 1, wherein said exopolysaccharide is produced by lactic acid bacteria.
3. The delivery system of claim 2, wherein said bacteria is selected from the group consisting of *Lactobacillus* strain R2C2, *Lactobacillus* strain Inix, *Lactobacillus* strain Es1, *Lactobacillus* strain K2, *Candida kefir* and *Candida norvegensis*.
4. The delivery system of claim 1, wherein said active molecule is selected from the group consisting of DNA, RNA, protein, peptide, peptidomimetic, virus, bacteria, nutraceutical product and pharmaceutical agent.
5. The delivery system of claim 4, wherein said pharmaceutical agent is selected from the group consisting of analgesic, anesthetic, antibiotic, anticancer, anti-inflammatory, and antiviral.
6. The delivery system of claim 5, wherein said anticancer agent is selected from the group consisting of alkylating agents, alkyl sulfonates, aziridines, ethylenimines, methylamelamines, acetogenins, camptothecin, bryostatin, callistatin, CC-1065, cryptophycins, dolastatin, duocarmycin, eleutherobin, pancratistatin, sarcodictyin, spongistatin, nitrogen mustards, nitrosureas, antibiotics, anti-metabolites, folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate, purine analogs, pyrimidine analogs, androgens, anti-adrenals, folic acid replenisher, aceglatone, aldophosphamide glycoside, aminolevulinic acid, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidamine, maytansinoids, mitoguazone, mitoxantrone, mopidamol, nitracrine, pentostatin, phenamet, pirarubicin, podophyllinic acid, 2-ethylhydrazide, procarbazine, PSK.RTM., razoxane,

rhizoxin, sizofiran, spirogermanium, tenuazonic acid, triaziquone, 2, 2',2"-trichlorotriethylamine, trichothecenes, urethan, vindesine, dacarbazine, mannomustine, mitobronitol, mitolactol, pipobroman, gacytosine, arabinoside, thiotepa, taxanes, chlorambucil, gemcitabine, 6-thioguanine, mercaptopurine, methotrexate, platinum, vinblastine, platinum, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, navelbin, novantrone, teniposide, daunomycin, aminopterin, xeloda, ibandronate, CPT-11, topoisomerase inhibitor RFS 2000, difluoromethylomithine, retinoic acid, capecitabine, anti-hormonal agents that act to regulate or inhibiting hormone action in hormonal dependent cancers.

7. The delivery system of claim 6, wherein said anti-hormonal agent is an anti-estrogens or an anti-androgens selected from the group consisting of flutamide, nilutamide, bicalutamide, leuprolide, and goserelin, and pharmaceutically acceptable salts, acids or derivatives thereof.

8. The delivery system of claim 6, wherein said alkylating agents is selected from the group consisting of thiotepa and cyclophosphamide (CYTOXAN™).

9. The delivery system of claim 6, wherein said alkyl sulfonates is selected from the group consisting of busulfan, improsulfan and piposulfan.

10. The delivery system of claim 6, wherein said aziridines is selected from the group consisting of benzodopa, carboquone, meturedopa, and uredopa.

11. The delivery system of claim 6, wherein said methylamelamines is selected from the group consisting of altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine.

12. The delivery system of claim 6, wherein said acetogenins is selected from the group consisting of bullatacin and bullatacinone.

13. The delivery system of claim 6, wherein said camptothecin is the synthetic analogue topotecan.

- 20 -

14. The delivery system of claim 6, wherein said CC-1065 is selected from the group consisting of adozelesin, carzelesin and bizelesin synthetic analogues thereof.
15. The delivery system of claim 6, wherein said cryptophycins is selected from the group consisting of cryptophycin 1 and cryptophycin 8.
16. The delivery system of claim 6, wherein said duocarmycin is selected from the group consisting of KW-2189 and CBI-TMI.
17. The delivery system of claim 6, wherein said nitrogen mustards is selected from the group consisting of chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide and uracil mustard.
18. The delivery system of claim 6, wherein said nitrosureas is selected from the group consisting of carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine.
19. The delivery system of claim 6, wherein said anti-metabolites is selected from methotrexate and 5-fluorouracil (5-FU).
20. The delivery system of claim 6, wherein said purine analogs is selected from the group consisting of fludarabine, 6-mercaptopurine, thiamiprine and thioguanine.
21. The delivery system of claim 6, wherein said pyrimidine analogs is selected from the group consisting of ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine and floxuridine.
22. The delivery system of claim 6, wherein said androgens is selected from the group consisting of calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone.
23. The delivery system of claim 6, wherein said anti-adrenals is selected from the group consisting of aminoglutethimide, mitotane and trilostane.
24. The delivery system of claim 5, wherein said antibiotics is selected from the group consisting of enediyne antibiotics, aclacinomysins,

actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, poffiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin.

25. The delivery system of claim 24, wherein said enediyne antibiotics is selected from the group consisting of calicheamicin, dynemicin, esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores.

26. The delivery system of claim 25, wherein said calicheamicin is selected from the group consisting of calicheamicin γ_1^I and calicheamicin θ_{1L} .

27. The delivery system of claim 25, wherein said dynemicin is dynemicin A.

28. The delivery system of claim 1, wherein said micelles are having a diameter varying from about 4 nanometers to about 400 nanometers.

29. A pharmaceutical composition comprising the delivery system of any one of claims 1 to 28 in association with a pharmaceutically acceptable carrier.

30. An immunomodulator composition comprising an immunomodulating amount of the delivery system of any one of claims 1 to 28 in association with a pharmaceutically acceptable carrier.

31. A method for delivering an active molecule to a patient comprising the step of administering the composition of claim 29 to said patient.

32. The method of claim 31, wherein said administering can be from a route selected from the group consisting out local, parenteral, peritoneal, mucosal, dermal, epidermal, subcutaneous, transdermal, intramuscular, nasal, oral, topical, vaginal, rectal, intra-ocular, intravenous, intra-arterial and by inhalation.

- 22 -

33. A method for inducing immunomodulation in a patient comprising the step of administering the composition of claim 30 to said patient.

34. The method of claim 33, wherein said administering can be from a route selected from the group consisting out local, parenteral, peritoneal, mucosal, dermal, epidermal, subcutaneous, transdermal, intramuscular, nasal, oral, topical, vaginal, rectal, intra-ocular, intravenous, intra-arterial and by inhalation.

35. Use of the composition of claim 29 for delivering an active molecule to a patient.

36. The use as claimed in claim 35, wherein said delivering can be from a route selected from the group consisting out local, parenteral, peritoneal, mucosal, dermal, epidermal, subcutaneous, transdermal, intramuscular, nasal, oral, topical, vaginal, rectal, intra-ocular, intravenous, intra-arterial and by inhalation.

37. Use of the composition of claim 30 for inducing immunomodulation in a patient.

38. The use as claimed in claim 37, wherein said delivering can be from a route selected from the group consisting out local, parenteral, peritoneal, mucosal, dermal, epidermal, subcutaneous, transdermal, intramuscular, nasal, oral, topical, vaginal, rectal, intra-ocular, intravenous, intra-arterial and by inhalation.

39. A method for producing the delivery system of claim 1, comprising the step of incubating exopolysaccharide in a suitable medium for a time sufficient to form micelle.

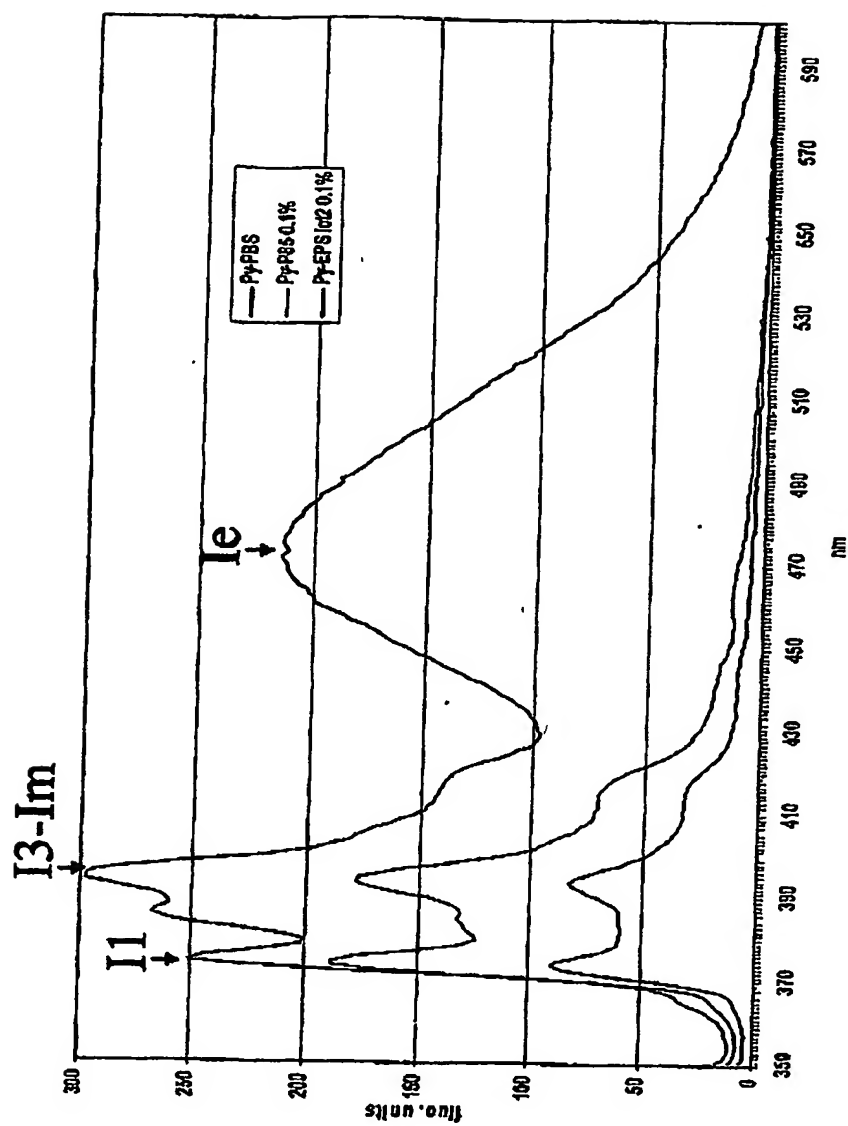


Fig. 1

DEC. 4. 2002 12:37PM

OGILVY MTL 514 288 8389

8382

P. 23/28

4-11-02

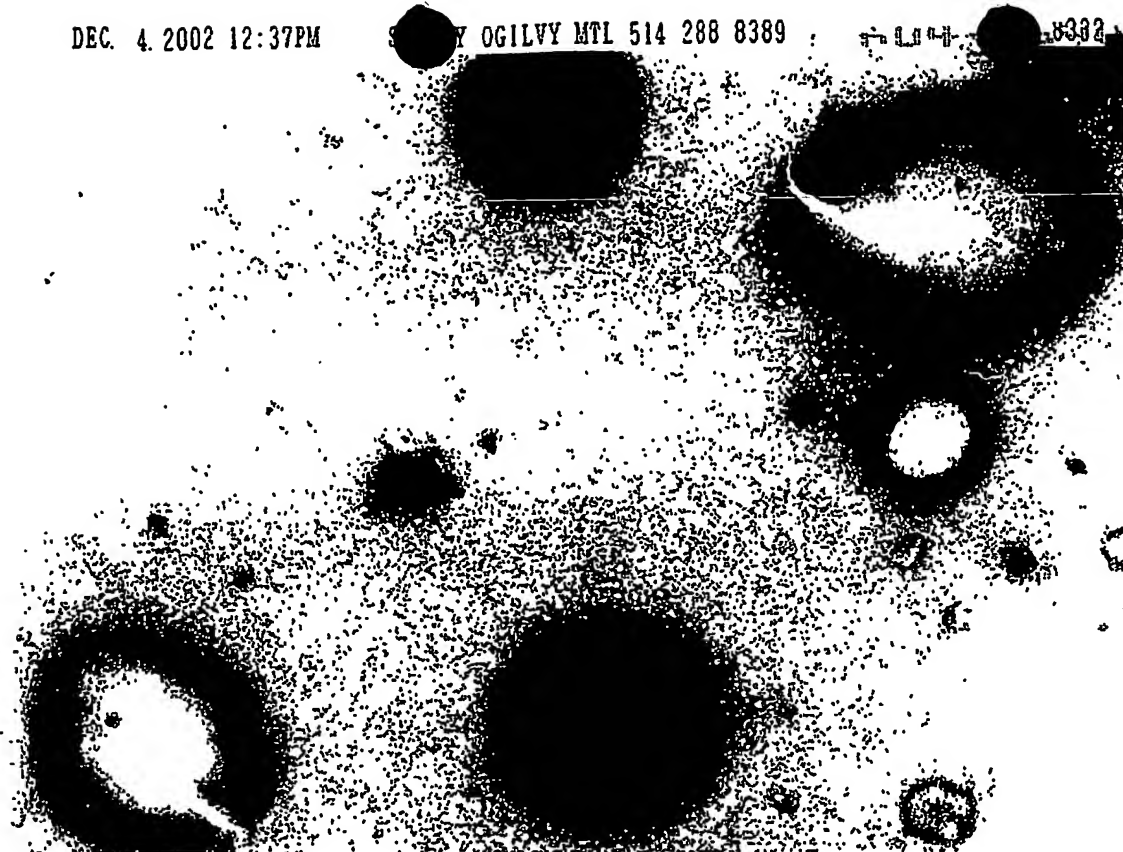


Fig. 2

DEC. 4. 2002 12:38PM

SV OGILVY MTL 514 288 8389

3332 P. 28/28

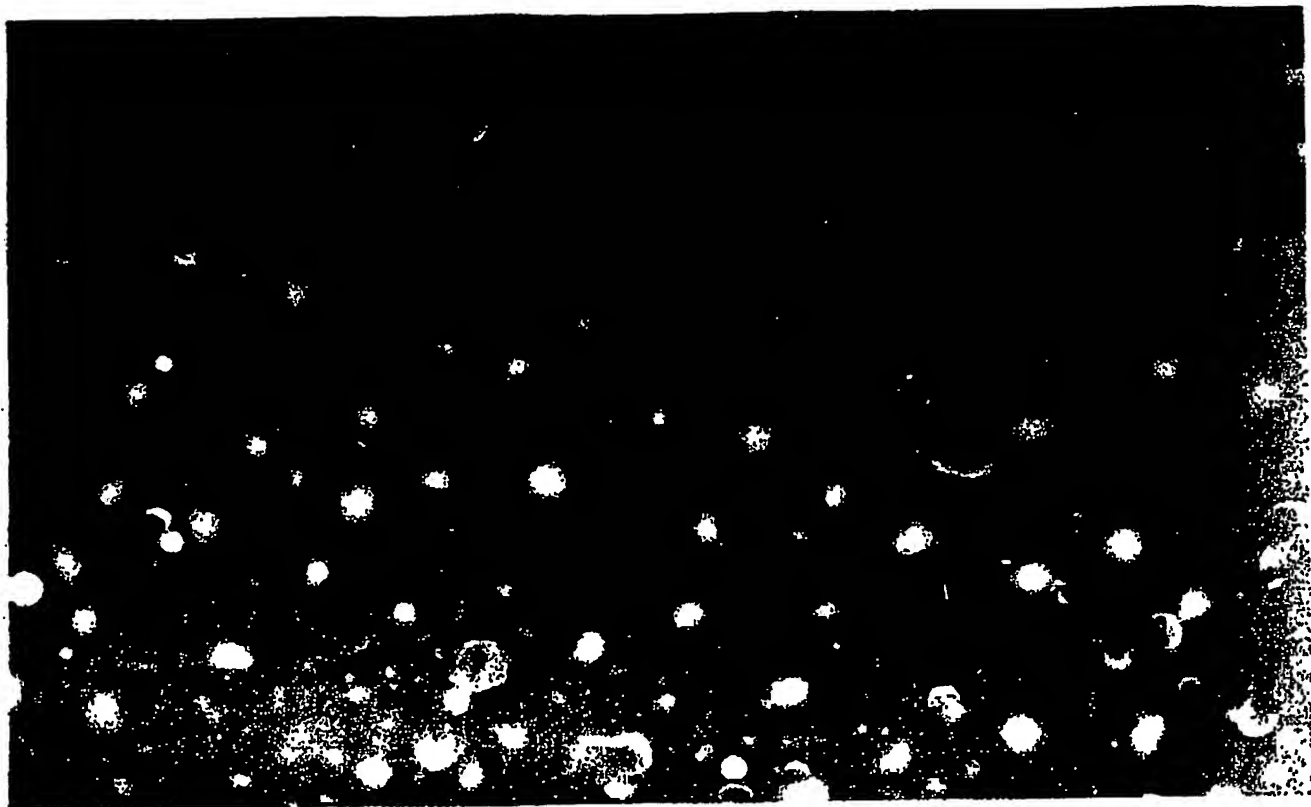


Fig. 3

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☒ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.